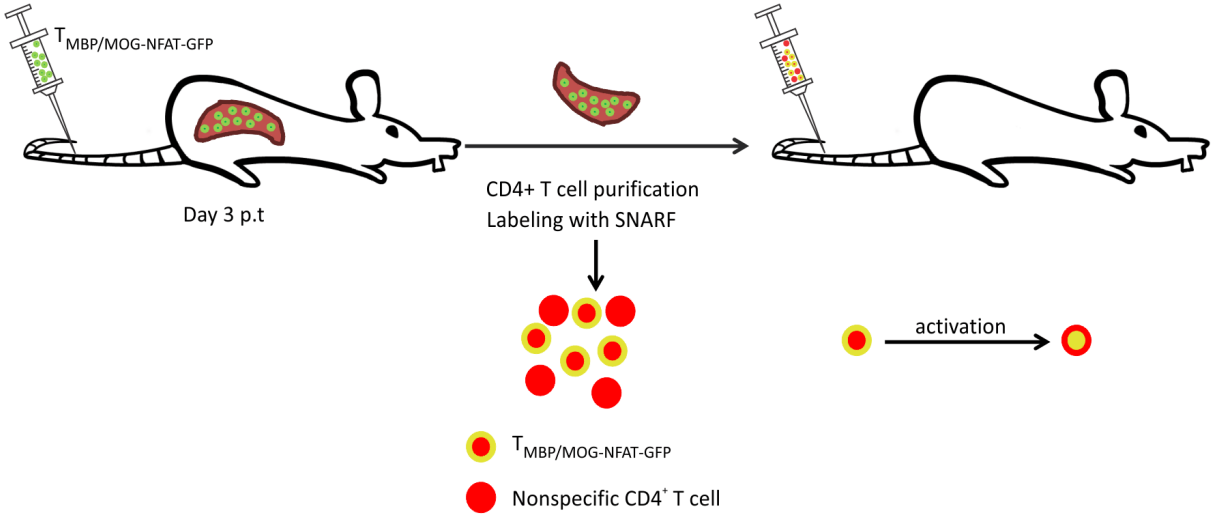
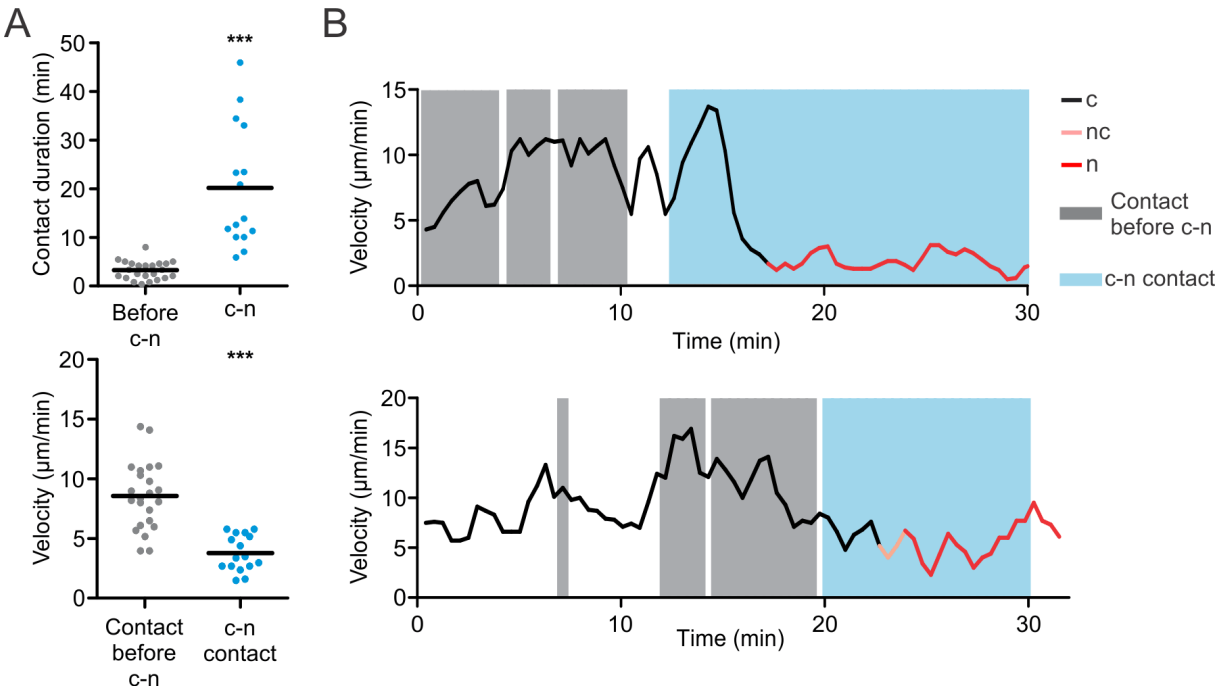
Supplemental Material



Supplemental Figure 1

Schematic overview of "migratory" $T_{MBP/MOG-NFAT-GFP}$ cells retransferred from the donor spleen in to WT recipient. Spleens were collected from donor animals at 3 days after the i.v. transfer of the $T_{MBP/MOG-NFAT-GFP}$ cells. $CD4^+$ T cells were purified from spleen, using a $CD4^+$ T cell isolation kit, and were labeled with SNARF. This mixed population of SNARF-labeled antigen-nonspecific (no ΔNFAT-GFP expression) single positive (red), and antigen- specific ΔNFAT-GFP and SNARF- labeled double- positive (red/green) cells, was injected in to WT recipients. Imaging was performed 12 h after re-transfer.



Supplemental Figure 2

Characterization of T/APC interactions prior and during $T_{MBP\text{-}NFAT\text{-}GFP}$ cell activation. (**A**) Contact duration, and T cell velocity during contact, was quantified for "non activating" (before c-n) and "activating" (c-n) contacts. Duration of activating (c-n) contacts was taken from Figure 5 B and C, and plotted for better comparison. Results are sum of five videos from three independent experiments. (**B**) Instantaneous velocity (averaged over three time points) for two representative $T_{MBP\text{-}NFAT\text{-}GFP}$ cells, making various "non-activating" (before c-n) contacts with APCs and finally making the "activating" one (c-n). Line color indicates ΔNFAT-GFP subcellular localization, and background color indicates T cell/APC interaction. ***p<0.0001(Nonparametric T test - Man Whitney)

Legends for supplemental Videos

Supplemental Video 1

In vitro ΔNFAT-GFP translocation from cytosol to nucleus and back upon ionomycin stimulation.

Resting in vitro $T_{MBP\text{-}NFAT\text{-}GFP}$ cells after ionomycin application and ionomycin wash out. Bright field images (grey) were overlaid with pseudo color images depicting GFP intensity distribution (from blue - low intensity, to red - high intensity). Note that ΔNFAT-GFP is translocated from cytosol to nucleus minutes upon ionomycin application while the reverse process took more than one hour after ionomycin wash out. The relative time after the start of acquisitions is indicated. Scale bar: 10 µm. Part of the movie before and after ionomycin application plays at 4 frames/s rate, while the part after washing plays at 10 frames/s rate.

Supplemental Video 2

Crawling T cells display cytosolic ΔNFAT-GFP.

$T_{MBP\text{-}NFAT\text{-}GFP}$ (green) cells crawling on the intraluminal surface of the blood vessel (red). The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 5 frames/s rate.

Supplemental Video 3

Rolling T cells have cytosolic ΔNFAT-GFP.

$T_{MBP\text{-}NFAT\text{-}GFP}$ (green) rolling within the blood vessel (red). Individual fast rolling cells appear repeatedly in single frame and are rounded. The frame with a rolling cell is stopped for a better visualization. The white arrowheads indicate the cells of interest. The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 5 frames/s rate.

Supplemental Video 4

Representative T_{MOG}/APC contacts.

The $T_{MOG-GFP}$ cells (green) were imaged together with the APCs (red) labeled with the intrathecal injection of Texas Red dextran. The trajectories of the T cell movements are overlaid. The white lines indicate the T cells while in contact with an APC; the blue lines denote when T cell is free. The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 5 frames/s rate.

Supplemental Video 5

Representative T_{MBP}/APC contacts.

The $T_{MBP-GFP}$ cells (green) were imaged together with the APCs (red) labeled with the intrathecal injection of Texas Red dextran. The trajectories of the T cell movement are overlaid. The white lines indicate the T cells while in contact with an APC; the blue lines denote when T cell is free. The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 5 frames/s rate.

Supplemental Video 6

SNARF-labeled $T_{MBP/MOG-NFAT-GFP}$ cells (red/green) in CNS meninges.

SNARF-labeled $T_{MBP-NFAT-GFP}$ (left) and $T_{MOG-NFAT-GFP}$ (right). The white arrows indicate the cells with a cytosolic localization of ΔNFAT-GFP (c) and the red arrow indicates cell with nuclear ΔNFAT-GFP (n); the light red arrow indicates the cell with a nuclear/cytosolic (nc) localization of ΔNFAT-GFP. Note that the $T_{MBP-NFAT-GFP}$ cells have c, n and nc ΔNFAT-GFP patterns, with one cell translocating ΔNFAT-GFP from the cytosol to the nucleus, whereas all of the $T_{MOG-NFAT-GFP}$ cells show only cytosolic ΔNFAT-GFP. The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 5 frames/s rate.

Supplemental Video 7

T cell velocity correlates with ΔNFAT-GFP localization.

Representative video of a SNARF-labeled $T_{MBP\text{-}NFAT\text{-}GFP}$ cell (red/green) showing translocation of ΔNFAT-GFP from cytosol to nucleus and diffusion to the cytosol and nucleus (nc) thereafter, is shown in the upper part. The instantaneous velocity of the cell (averaged over three time points) is depicted in the lower portion. The dark and light red background of the graph indicate the time when ΔNFAT-GFP is being localized in the nucleus (n) and when ΔNFAT-GFP is diffused in both the cytosol and nucleus (nc) respectively. Note that the cell is slowing down when ΔNFAT-GFP is located in nucleus. The relative time after the start of acquisition is indicated. Scale bar: 10 μm. Movie plays at 5 frames/s rate.

Supplemental Video 8

$T_{MBP\text{-}NFAT\text{-}GFP}$ cells translocate ΔNFAT-GFP from the cytosol to nucleus upon contact with a local APC.

Two SNARF-labeled $T_{MBP\text{-}NFAT\text{-}GFP}$ (red/green) cells becoming activated (translocation of ΔNFAT-GFP from the cytosol to nucleus) upon contact with a local APC (cyan, speculated outline indicated with a dotted line). The closed arrowheads indicate the T cell/APC interactions leading to activation, and the open arrows indicate the cells of interest. Note that both cells are receiving an activation signal from the same APC. The top right inserts show the cell of interest at a higher magnification in the red and green channels only. The relative time after the start of acquisition is indicated. Scale bar: 10 μm. Movie plays at 7 frames/s rate.

Supplemental Video 9

$T_{MBP\text{-}NFAT\text{-}GFP}$ cell translocate ΔNFAT-GFP from the nucleus back to the cytosol after detaching from a local APC.

A SNARF-labeled $T_{MBP\text{-}NFAT\text{-}GFP}$ (red/green) cell with nuclear ΔNFAT-GFP contacting a local APC (cyan); ΔNFAT-GFP is slowly translocated back to the cytosol after the cell detaches from the APC. The closed arrowheads indicate the T cell/APC interactions, and the open arrows indicate the cell of interest. The top right corner inserts show the cell of interest at a higher magnification in the red and green channels only. The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 10 frames/s rate.

Supplemental Video 10

Local application of MOG leads to nuclear ΔNFAT-GFP translocation in $T_{MOG\text{-}NFAT\text{-}GFP}$ cells.

SNARF-labeled $T_{MOG\text{-}NFAT\text{-}GFP}$ (red/green) cells before and after the local application of MOG and their interactions with local APCs (blue). The closed arrowheads indicate the T cell/APC interactions leading to activation, and the open arrows indicate the cells of interest. The relative time after the start of acquisition is indicated. Scale bar: 10 µm. Movie plays at 5 frames/s rate.